Anti-apoptotic activity of the glutathione peroxidase homologue encoded by HIV-1

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Abstract:

The third reading frame of the envelope gene from HIV-1 codes for a protein homologous to the human selenoprotein glutathione peroxidase (GPX). Cells stably or transiently transfected with a HIV-1 GPX construct are protected against the loss of the mitochondrial transmembrane potential and subsequent cell death induced by exogenous reactive oxygen species (ROS) as well as mitochondrion-generated ROS. However, HIV-1 GPX does not confer a general apoptosis resistance, because HIV-1 GPX-transfected cells were not protected against cell death induced by staurosporine or oligomycin. The inhibition of cell death induced by the ROS donor *tert*-butylhydroperoxide was also observed in cells depleted from endogenous glutathione (GSH), suggesting that GSH is not the sole electron acceptor for HIV-1 GPX. Clinical HIV-1 isolates from long-term non-progressors (untreated patients with diagnosed HIV-1 infection for >10 years, with CD4 T cell count of >500 cells/mm^3) mostly possess an intact GPX gene (with only 18% of loss-of-function mutations), while HIV-1 isolates from patients developing AIDS contain non-functional GPX mutants in 9 out of 17 cases (53%). Altogether, these data suggest that HIV-1 GPX possesses a cytoprotective, pathophysiologically relevant function.

Keywords: glutathione | mitochondria | selenium

Article:

Abbreviations: BSO: buthionine sulfoximine; $\Delta\Psi_m$: mitochondrial transmembrane potential; $DiOC_6(3)$: 3,3′ dihexyloxacarbocyanine iodide; GSH: glutathione; GPX: glutathione peroxidase; HE: hydroethidine; HIV-1: human immunodeficiency virus 1; PI: propidium iodide; ROS: reactive oxygen species; STS: staurosporine; *t*-BHP: *tert*-butylhydroperoxide.

Introduction

Human immunodeficiency virus-1 (HIV-1) merciless exploits the host cell to guarantee its replication and propagation. One of the major pathways targeted by HIV-1 is the apoptotic machinery.[1–3] Indeed, the temporary suppression of apoptosis of HIV-1 infected cells is a *conditio sine qua non* for HIV-1 to replicate and to constitute the viral reservoir. In contrast, the induction of apoptosis may ultimately participate in viral spreading and certainly contributes to the subversion of the immune system by HIV-1.[1–3] An increased apoptotic turnover of immune cells constitutes a hallmark of HIV-1 infection. Thus, an enhanced propensity of $CD4^+$ lymphocytes to undergo apoptosis *in vitro* is associated with progressive disease,[4–6] and this laboratory parameter is one of the first to improve when patients are treated successfully with highly active anti-retroviral therapy (HAART).[7–11] Similarly, patients developing the acquired immunodeficiency syndrome (AIDS) manifest an increased percentage of circulating $CD4^+$ T cells with a reduced mitochondrial transmembrane potential ($\Delta\Psi_m$),[12,13] one of the hallmarks of imminent or ongoing apoptosis.[14–17] In contrast, so-called long-term non-progressors (LTNP), patients not receiving any anti-retroviral therapy who do not develop lymphodepletion (CD4 T cell count of >500 cells mm^3*)* within 10 years after primoinfection, exhibit a low propensity of T cells to undergo apoptosis *in vitro*[18] and a normal, low percentage of circulating cells with a reduced $\Delta\Psi_m$ .13 This phenotype correlates with a specific loss-of-function mutation of the HIV-1 encoded proapoptotic protein Vpr,[19] a mutation that is found in 81% of LTNP.[18] Thus viral mutations in apoptosis-regulatory proteins may determine HIV-1 virulence and, ultimately, the patient's fate.[20]

HIV-1-infected cells manifest a shift in the redox balance, with an increased production of reactive oxygen species,[21,22] as well as a depletion of anti-oxidant metabolites such as glutathione (GSH),[21] a condition which is likely to augment the propensity of cells to undergo apoptosis.[23–25] Similarly, circulating lymphocytes from HIV-1 infected donors tend to produce elevated levels of reactive oxygen species (ROS),[26] a fraction of which, at least, is produced by deregulated mitochondria.[12] Reduced circulating levels of GSH indicate a poor clinical prognosis,[27,28] and several authors advocate the therapeutic supplementation of anti-oxidant compounds [29] including N-acetyl cysteine, a GSH precursor [28,30] and selenium (Se).[31]

Recently, it has been reported that HIV-1 would encode a GSH peroxidase (GPX),[32] an enzyme that detoxifies peroxide radicals while oxidizing glutathione. Thus, the third reading frame of the envelope (Env) gene (which itself is largely pro-apoptotic)[33] encodes a putative selenoprotein, that is a protein which contains a catalytic selenocysteine (Sec) residue.[32] In addition, HIV-GPX bears structural similarities with mammalian GPX, including the catalytic triad selenocysteine (U), Gln (Q) and Trp (W), as well as the overall GPX fold, as deduced from computerized calculations.[32] On theoretical grounds, the existence of such an HIV-1 GPX may explain the tendency of host-cell selenoproteins to be underexpressed, as a consequence of competition for the oligoelement Se.[34,35] Since such selenoproteins are mostly anti-oxidant enzymes (in particular GSH peroxidases and thioredoxin reductases), this would lead to reduction of the anti-oxidant defense of the host cell organism.[35] Conversely, it is possible that HIV-1 GPX might exert a catalytic activity that itself enhances the anti-oxidant system of the host cell,32 as this has been reported for a GPX encoded by molluscum conagiosum virus.[36]

Intrigued by these possibilities, we decided to explore the putative apoptosis-modulatory function of HIV-1 GPX. Here, we show that mammalian cells manipulated to express the HIV-1

GPX gene exhibit a relative resistance against exogenous ROS as well as ROS produced by mitochondria, leading to a selective apoptosis resistance. Moreover, we show that viral isolates from patients with progressive disease carry HIV-1 strains with loss-of-function mutations in the GPX gene. These findings may have major implications for the pathophysiology of HIV-1 infection.

Materials and methods

Sequence analysis

HIV-1 sequences were obtained from the Los Alamos AIDS data base and were analyzed by using the BLAST program (NCBI).

Cell culture, transfection, and apoptosis induction

The MDCK (Madi Darbin Canine Kidney) cell line stably transfected with the histidine tagged HIV-1 Gpx protein (pHGD), as well as control cells transfected with vector only (pC2) were described previously.[32] The nonmanipulated MDCK cell line was obtained from the American Type Culture Collection (ATCC). All cell types were grown under 5% CO2 at 37°C in DMEM medium supplemented with 2 mM glutamine, 10% newborn calf serum, 1 mM pyruvate, 10 mM Hepes, 100 U/ml pencillin/streptomycin and 5 μg/ml gentamycin (GIBCO). MDCK cells were transfected by using Polyfect reagent (Quiagen), on six-well plates, with each well seeded with 2.5×10^5 cells and 5 μg of plasmid DNA. 24 hours post-transfection, transfected cells were selected for antibiotic resistance to G418 at 400 μg/ml, by changing G418-containing medium every 3 days for 21–30 days.

Induction and quantification of apoptosis

For cell death induction, 5×10^4 cell were grown overnight on six-well plates and treated with various doses of *ter*-butylhydroperoxide, stauroporine, antimycin A, oligomycin (Sigma) during an overnight incubation at 37°C. BSO was used at a concentration of 100 μM to deplete endogenous GSH levels, 24 h before apoptosis induction. All chemicals reagents were purchased from Sigma. The following fluorochromes (Molecular Probes) were employed to assess apoptosis-associated changes by cytofluorometry on a FACS Vantage (Becton Dickonson), while gating the forward and the side scatters on viable cells: 3,3′ dihexyloxacarbocyanine iodide ($DiOC_6(3)$, 40 nM; 15 min at 37°C) or tetramethylrhodamine ethyl ester (TMRE, 150 nM; 15 min at 37°C) for $\Delta\Psi_m$ quantification, propidium iodide (PI, 1 μg/ml) for the determination cell viability.[37] The generation of ROS was monitored with hydroethidine (HE, 2 μM; 15 min at 37°C). Labeling with Annexin V-FITC conjugate (5 μl; Pharmingen, San Diego, CA) to assess of the aberrant phosphatidylserine (PS) residues exposure, was performed as described.[37] The content in free thiols (mainly GSH) was determined using monochlorobimane (MCB, 50 μM).[23]

Construction of the pIRES-HGD

Six histidine residues have been incorporated between the Met start codon and the Gly residue that is at the N-terminal end of the putative GPX sequence in order to obtain a histidine tagged

HIV-l GPX. Furthermore, since the selenocysteine insertion in eukaryotic selenoproteins involves an RNA stem-loop structure called a selenocysteine insertion sequence (SECIS) element in the 3′-untranslated region of the mRNA, the His-HIV-l GPX has been fused to the SECIS element of the rat 5′-deiodinase (5′-DI).[32] This His-HIV-l GPX-5′-DI SECIS construct (termed HGD) was subcloned into the pEGFPC1 vector. The HGD insert was exised from pEGFPC1 as an NheI-BamHI fragment and ligated into the pIRES-EGFP (Clontech) vector digested by the same enzymes to obtain pIRES-HGD. The quality of DNA subcloning was confirmed by DNA sequencing. The *E. coli* DH5 α strain was used to propagate various plasmids and their derivatives.

RNA isolation and RT-PCR reaction

1×10^6 cell stably transfected with pIRES or pIRES-HGD were grown during 2 days in the presence or absence of selenium (20 nM). Cells were washed twice in PBS and RNA were purified with the RNeasy extraction kit (QIAGEN). After a reverse transcriptase polymerase chain reaction (RT-PCR) amplification using the following primers: 5′- primer 5′-CGGAATTCGTGCATCATCATCAT-3′ and 3′-primer: 5′-GCGGATCCCGGATTTTAATCGTT-3′, corresponding to the HGD sequence (Oligo Gpx) and the 3′-primer: 5′-ATGGTGAGCAAGGGCGAGGAG-3′ as control (Oligo Co). Primers corresponding to the β-Actine RNA present in the RT-PCR kit were used as control for the efficacity of the reaction. All amplified DNA fragments were visualized by agarose gel electrophoresis.

Results and discussion

Conservation of the HIV-1 GPX gene among clinical HIV-1 isolates

The third reading frame of the Env gene codes for a putative GPX selenoprotein.[32] HIV-1 GPX amino acid sequences obtained from primary HIV-1 isolates, either from patients with progressive disease or from long-term non-progressors (LTNP) were obtained from public data bases and aligned. As shown in Figure 1, the HIV-1 GPX gene was well conserved among most long-term nonprogressors (LTNP), that is HIV-1 infected individuals which, without any treatment, remain essentially disease-free with normal $CD4^+$ counts. Only in two of the eleven LTNP isolates, stop codons were found to truncate the protein. In contrast, 59% of clinical isolates obtained from progressors manifested either a substitution of the critical HIV-1 GPX selenocysteine residue (marked as “U” within the R1 domain) and/or a truncation due to stop codons in the N-terminus of the protein, within the functionally important R2 region, or at the N-terminus of the R3 region, meaning that the enzymatic function of the protein must be lost (Figure 1). Of note, all but two of the GPX genes derived from patients developing AIDS (15 among 17) exhibited non-conservative mutations in the R1 region (32 non-conservative replacements among 160 residues), while none (0 among 100 residues) of the R1 regions from LTNPs was mutated (Figure 1). Thus, the HIV-1 GPX gene appears to be conserved in laboratory strains of HIV-1, as well as in LTNP isolates, while loss-of-mutations affect a majority of HIV isolates from patients with progressive disease.

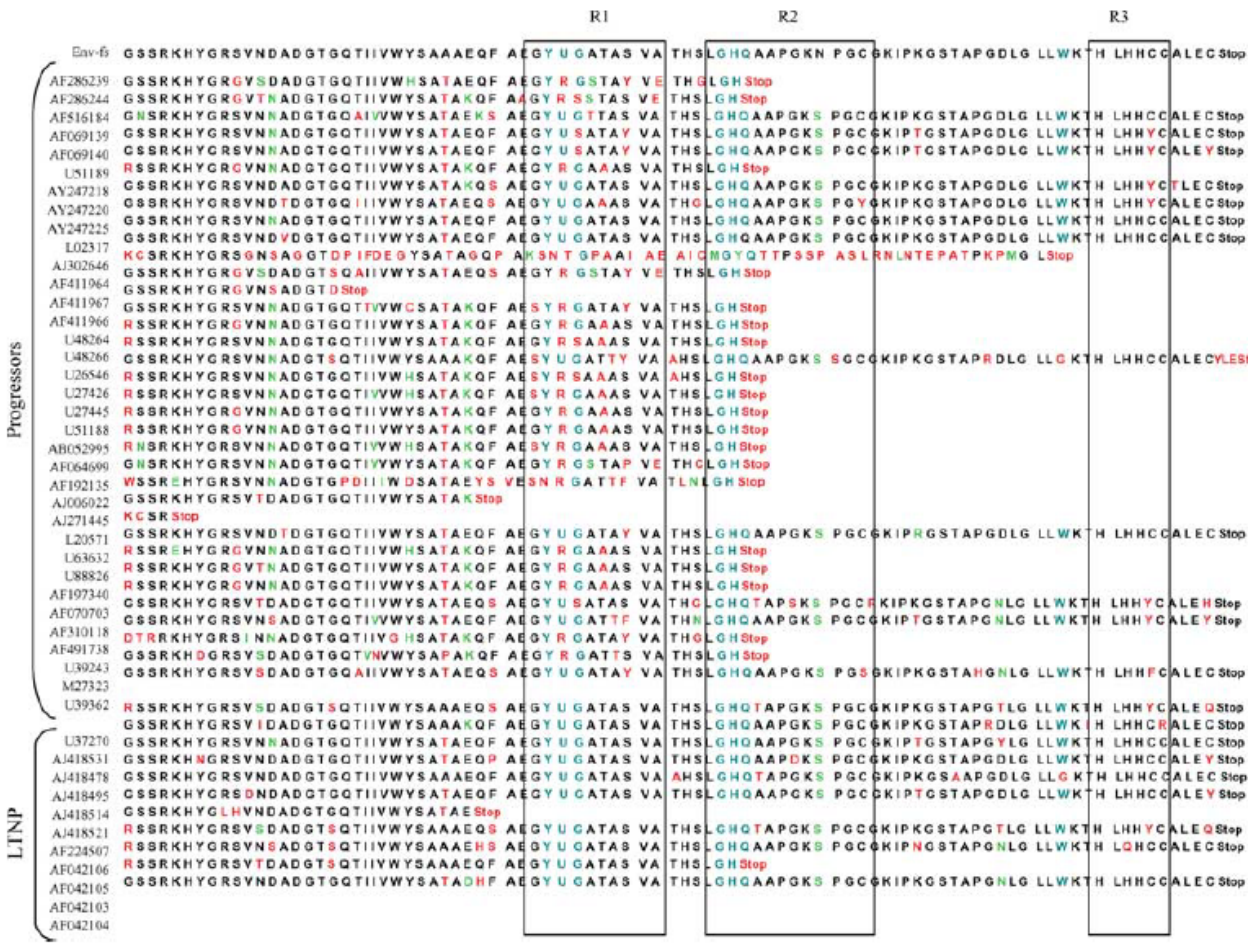

Figure 1. Comparison of GPX sequences in different HIV-1 isolates. The open reading frame encoding for the putative GPX protein was located in the third reading frame of the Env gene from different HIV-1 isolates, obtained from patients that develop AIDS (progressors) as well as from long-term non-progressors (LTNP). R1 (boxed) contains the selenocysteine residue (denoted as "U"), while R2 and R3 contain additional conserved active-site regions. The Env-fs sequence is the consensus GPX sequence from HIV-1^{LAI} reported in Ref. [32]. Note that several GPX proteins deduced from virulent HIV-1 isolated have mutated the selenocysteine-encoding UGA to arginine (AGA) and possess a stop codon leading to a truncation that is incompatible with the redox function of the enzyme. Conservative amino acid changes are depicted in green, while non-conservative changes are labeled in red. The GenBank accession codes of each HIV-1 isolate are indicated.

Selective apoptosis resistance conferred by stably expressed HIV-1 GPX

Cells stably transfected with an HIV-1 GPX construct engineered to contain the selenocysteine insertion sequence (SECIS) element in the 3′ untranslated region[32] (pHGD cells), as well as vector-only control cells (pC2 cells), were cultured in the absence or presence of the trace element Se (provided as sodium selenite) and exposed to the general apoptosis inducer staurosporine (STS) or the ROS donor *tert*-butylhydroperoxide (*t*-BHP), followed by determination of several parameters of apoptosis: the dissipation of the $\Delta\Psi_m$ (quantified with the $\Delta\Psi_m$ -sensitive dye $DiOC_6(3)$), the cell-autonomous generation of ROS (quantified with hydroethidine [HE], a non-fluorescent plasma membrane-permeable compound that is oxidized by ROS to ethidium [Eth], a fluorescent, hydrophilic compound that is trapped in the cell) (Figure 2A), the exposure of phosphatidylserine moieties on the plasma membrane surface

(quantified with Annexin V-FITC conjugates), and the loss of viability (quantified with the vital dye propidium iodide) (Figure 3A). Selenite conferred a relative resistance against *t*-BHP, in accord with its apoptosis-inhibitory effect.[38,39] Cells expressing HIV-1 GPX (pHGD) were more resistant against low doses of *t*-BHP than control cells, and this effect was more pronounced in the presence of Se than in its absence. Thus, a depletion of Se from endogenous selenoproteins cannot account for the apoptosis-modulatory effect of GPX. Neither Se nor GPX conferred any protection against STS-induced cell death (Figures 2 and 3). The selective HIV-1 GPX-mediated inhibition of *t*-BHP (but not STS)-induced cell death was detectable with all four read-outs of apoptosis induction ($\Delta\Psi_m$ loss and ROS overproduction in Figure 2, phosphatidylserine exposure and plasma membrane permeabilization in Figure 3).

Apoptosis resistance conferred by HIV-1 GPX in transient transfection experiments

In stably transfected cell lines, cloning artifacts or epigenetic phenomena with alterations in the transcriptome might influence the experimental outcome. We therefore decided to clone HIV-GPX into a pIRES vector that leads to the joint transcription/translation of HIV-1 GPX and green fluorescent protein (GFP) from the same mRNA species. As shown in Figure 4a, the addition of an external source of Se did not influence the level of GFP expression, suggesting that the stability of the HIV-1 GPX mRNA is not influenced by Se. FACS purification of cells transiently transfected with HIV-1 GPX plus GFP (and as a negative control, cells that only express GFP encoded by the pIRES vector) (Figure 4b), followed by RT-PCR revealed that the cells indeed expressed HIV-1 GPX mRNA (Figure 4c) that was unmutated (not shown). In transient transfection experiments, HIV-1 GPX did confer a significant protection against *t*-BHP-induced $\Delta\Psi_m$ loss (quantified with tetramethylrhodamine ester, TMRE, Figure 5a) and cell death (quantified with PI, Figure 5b). Thus, HIV-1 GPXis functionally relevant for cell survival in conditions of ROS-mediated stress.

HIV-1 GPX inhibits apoptosis induced by mitochondrion-generated ROS

The experiments described above involved the addition of an exogenous source of ROS. To investigate whether HIV-1 GPX also inhibits apoptosis induced by endogenous ROS (which are mostly produced by mitochondria), we took advantage of antimycin A (Ant A), an inhibitor of respiratory chain complex III that stimulates the production of ROS.[40] Addition of antimycin A led to a global shift in the production of HE-detectable ROS, which was not influenced by Se or HIV-1 GPX (Figure 6a). The antimycin A-induced $\Delta\Psi_m$ loss was not or only marginally influenced by Se, yet was strongly inhibited by HIV-1 GPX (Figure 6a and b). In sharp contrast, HIV-1 GPX did not protect against the cytotoxic effect of oligomycin, an inhibitor of complex V($F_{1/0}$ ATPase) that does not trigger ROS overproduction (Figure 6a and b). Altogether, these data indicate that HIV-1 GPX can protect cells against ROS produced from an endogenous source and confirm the selective apoptosis-modulatory function of HIV-1 GPX.

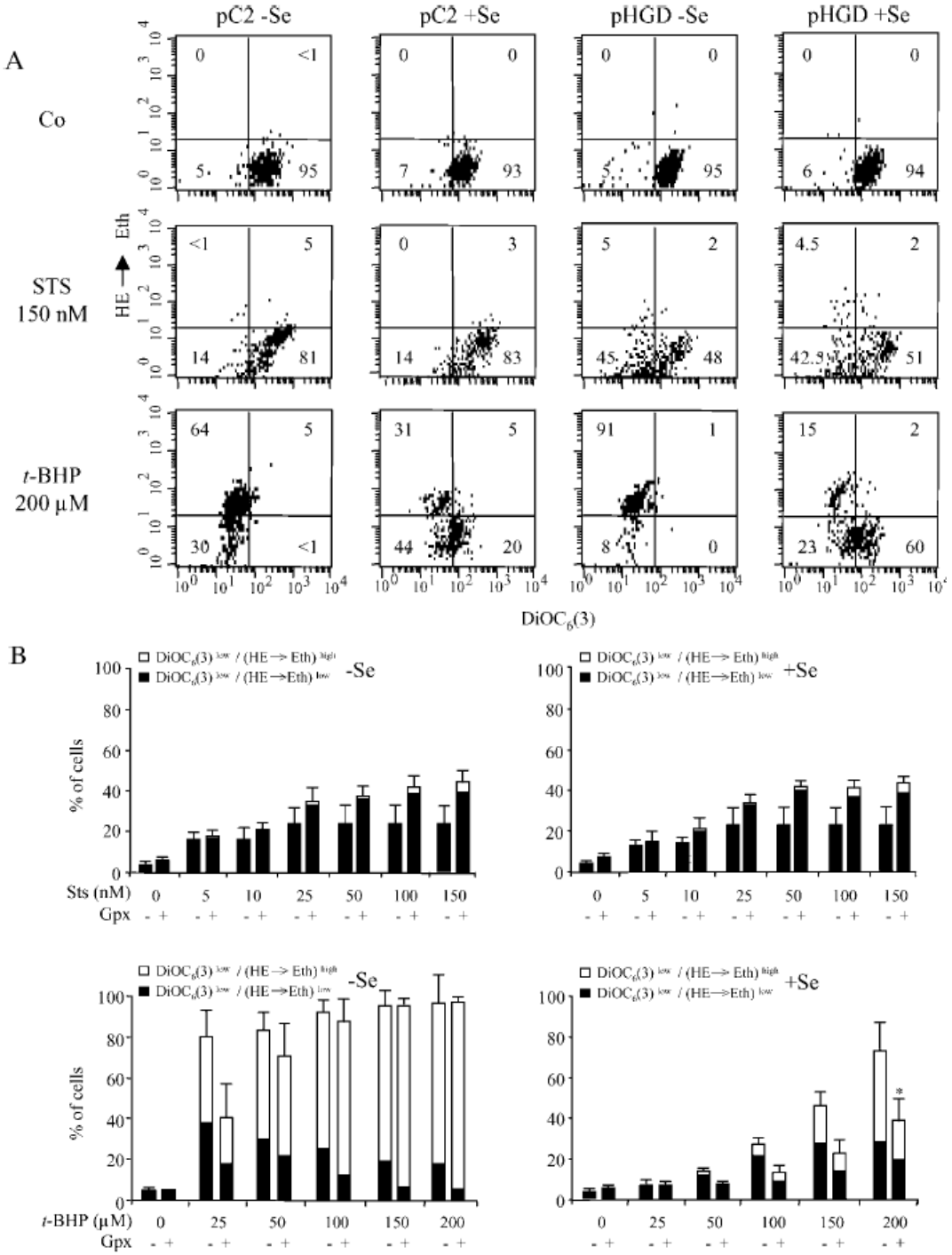

Figure 2. Anti-apoptotic action of GPX as assessed by evaluation of the mitochondrial transmembrane potential ($\Delta\Psi_m$). MDCK cell lines stably transfected with vector only (pC2) or with an HIV-1-encoding GPX construct (pHGD) were cultured in the absence (−Se) or presence (+Se) of 20 nM selenium, as well as the indicated doses of staurosporine (STS) and the ROS donor *tert*-butylhydroperoxide (*t*-BHP). Eighteen hours later cells were trypsinized and stained with a combination of two fluorochromes, namely $DiOC_6(3)$ (which is $\Delta\Psi_m$ -sensitive) and hydroethidine (HE, which is non-fluorescent, yet emits a fluorescence upon oxidation to ethidium, Eth), followed by cytofluorometric analysis. Original FACS diagrams are depicted in A. Note that the $(HE \rightarrow Eth)^{high}$ cells constitute a fraction of $DiOC_6(3)^{low}$ cells. Mean values of eight independent experiments (X±SEM) are shown in B. Arrows denote significant ($p < 0.05$) differences between cells tranfected with HIV-1 GPX or not.

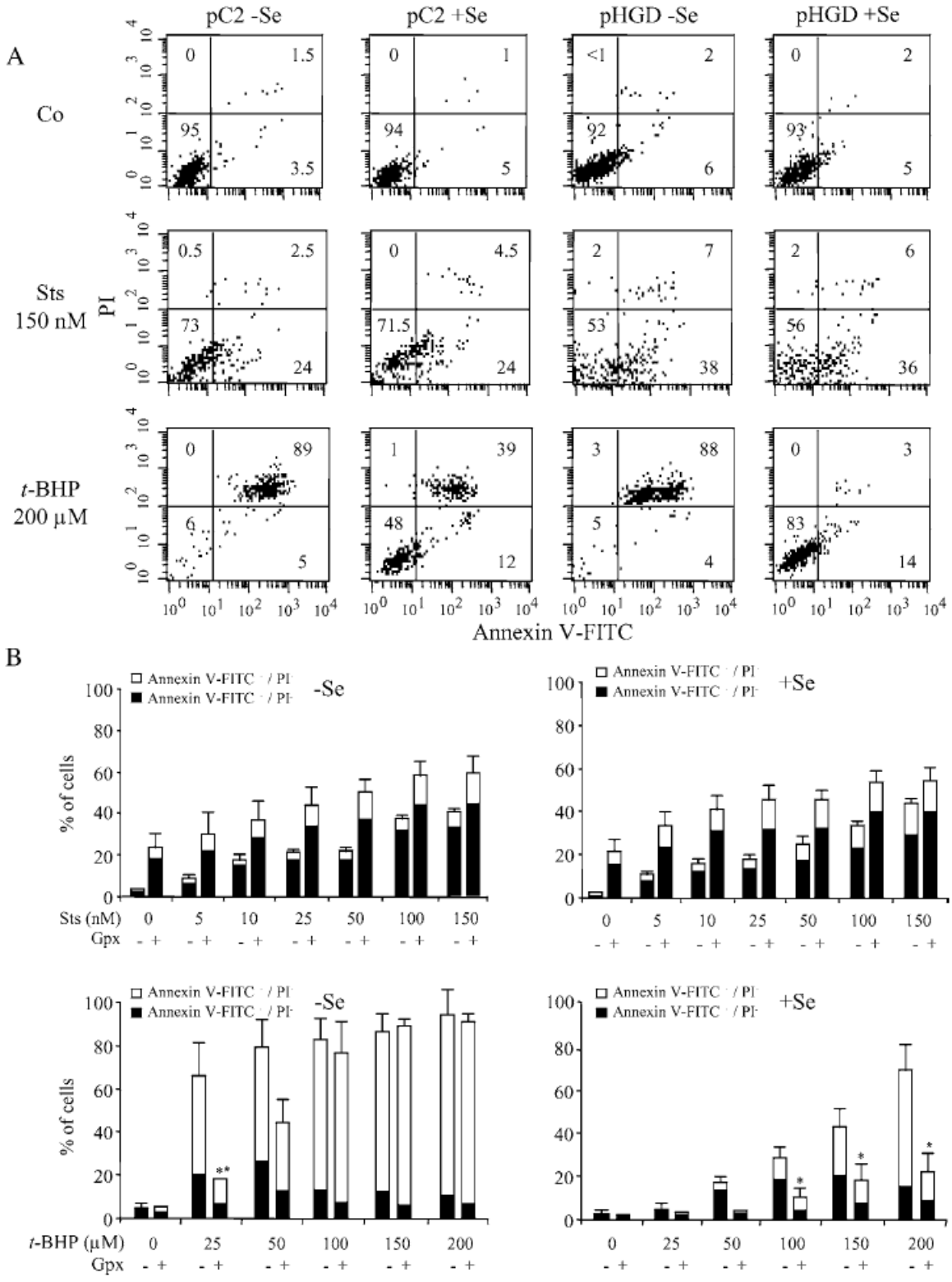

Figure 3. Anti-apoptotic action of GPX as assessed by assessment of phosphatidylserine exposure on the cell surface. The experimental setup was similar to that described in Figure 2, with the difference that apoptosis was assessed by staining with annexin V-FITC conjugates plus the vital dye propidium iodide. Original FACS pictograms are shown in A, while the pooled data from six independent experiments (X±SEM) are shown in B. Arrows denote significant ($p < 0.05$) differences between cells transfected with HIV-1 GPX or not.

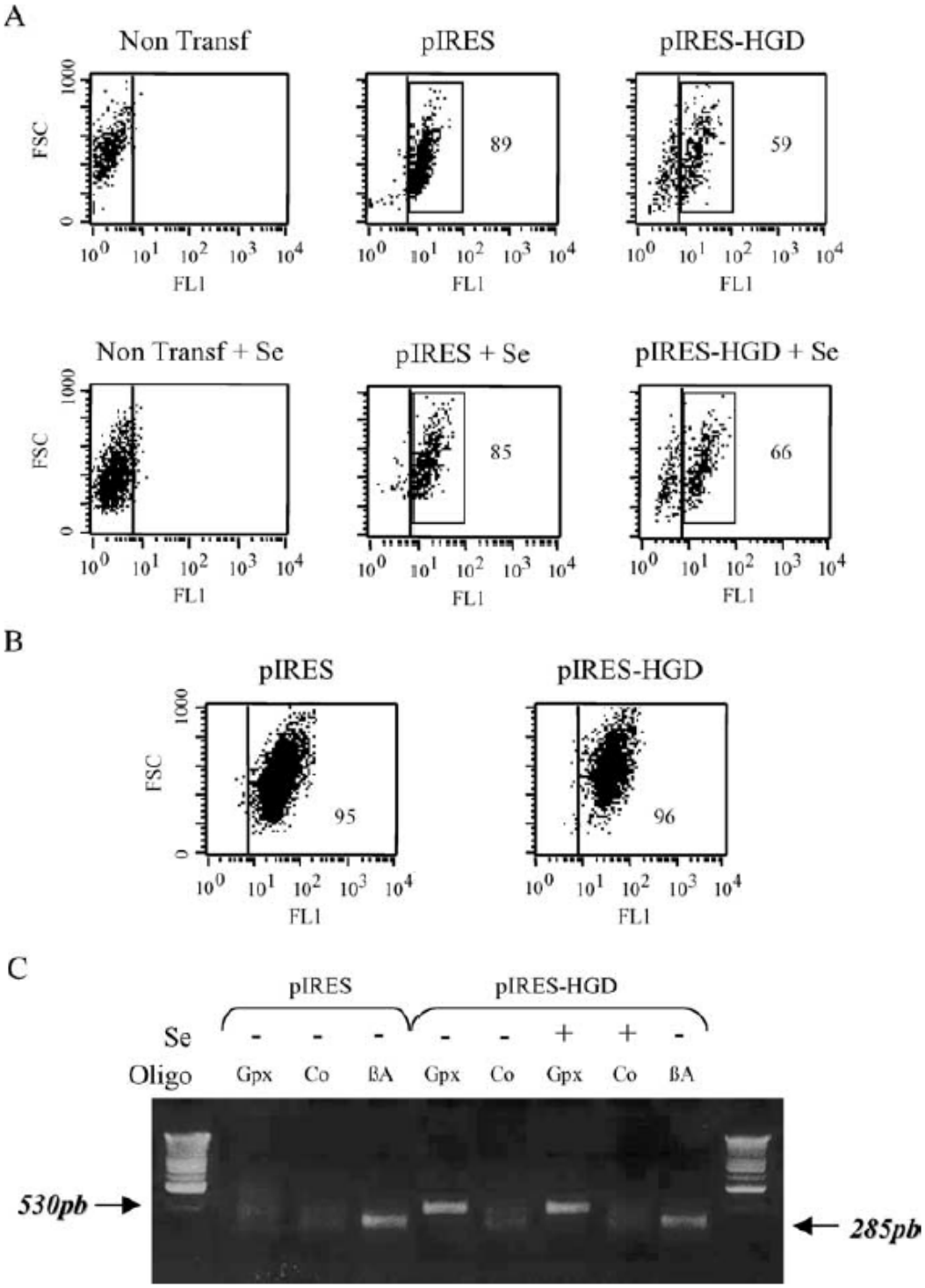

Figure 4. Characterization of MDCK cells transiently transfected with a vector coding for both GFP and HIV-1 GPX. MDCK were either mock transfected or transfected with a vector encoding GFP alone (pIRES) or a vector coding for both GFP and HIV-1 GPX (pIRESGPX). Cells were cultured either in the presence or in the absence of Se, followed by FACS analysis to detect GFP expression (in FL1), 48 h after transfection (A). Numbers refer to the percentage of cells positive for GFP. Cells found positive for GPF (gates in A) were FACS-purified and re-analyzed in the cytofluorometer to assess the purity of the population (B). Such FACS-purified cells transfected with either the pIRES or the pIRES-GPX construct, cultured in the absence or in the presence of Se, were subjected to RT-PCR analysis using suitable primers for the detection of HIV-1 GPX or β-actin.

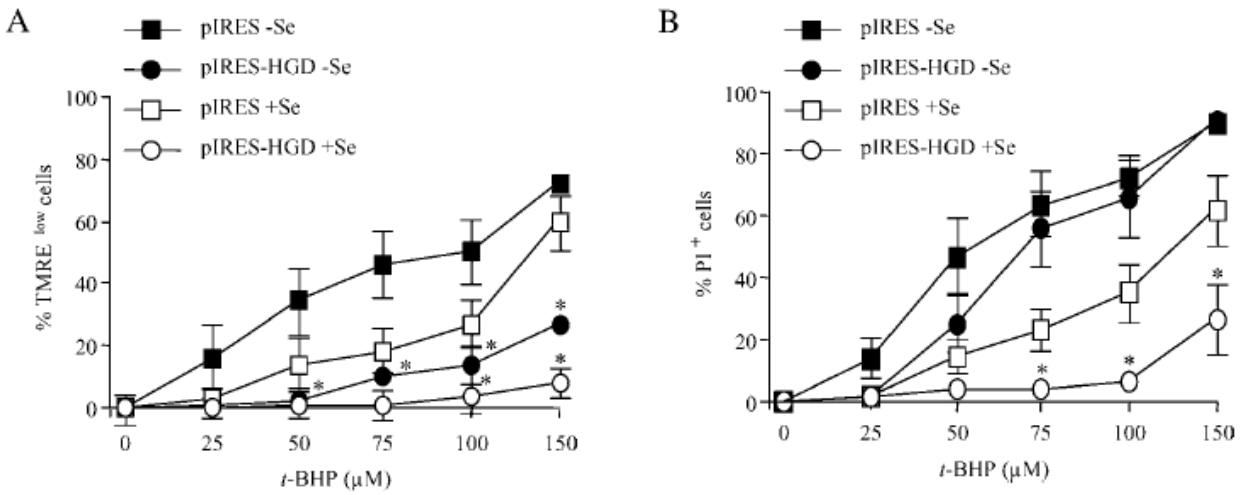

Figure 5. Transient expression of HIV-1 GPX confers resistance against *t*-BHP. MDCK cells transfected with the pIRES vector alone (which encodes GFP) or the pIRES-HGD construct (which encodes GFP plus HIV-1 GPX) were treated with the indicated concentrations of *t*-BHP, in the presence or absence of 20 nM Se. After overnight incubation the cells were stained with the $\Delta\Psi_m$ -sensitive dye TMRE or PI, and the frequency of cells exhibiting a low $\Delta\Psi_m$ (and hence a low TMRE fluorescence) or a permeable plasma membrane (and hence incorporation of PI) was determined while gating on the population of cells expressing GFP as in Figure 4. Results are mean values of five different experiments. Arrows denote significant ($p < 0.01$) differences between cells tranfected with pIRES-HGD or pIRES vector only.

GSH-independent anti-apoptotic potential of HIV-1 GPX

If reduced GSH was the unique electron acceptor of HIV-1 GPX, the protein should lose its anti-oxidant (and hence anti-apoptotic) function in conditions in which GSH is depleted. We therefore depleted GSH by pretreating pHGD and pC2 cells with buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, while controlling effective GSH depletion with monochlorobiman (MCB) staining (Figure 7c). As to be expected, GSH depletion enhanced the susceptibility of both pC2 and pHGD cells to *t*-BHP-induced $\Delta\Psi_m$ loss (Figure 7a) and cell death (Figure 7b). Surprisingly, however, even in the absence of endogenous GSH, pHGD cells remained relatively resistant (as compared to pC2 cells) against the $\Delta\Psi_m$ loss induced by to *t*-BHP. Thus, HIV-1 GPX protects against ROS-induced apoptosis in conditions in which GSH is depleted.

Concluding remarks

The data presented in this paper indicate that the putative HIV-1 GPX may well have an important anti-apoptotic function, conferring cytoprotection against exogenous or endogenous ROS. HIV-1 possesses several proteins which induce apoptosis via redox-sensitive effects. Thus, Tat has been shown to interfere with the expression and/or function of the potent ROS detoxifying enzyme superoxide dismutase-2,[41] and Vpr can perturb mitochondrial function by inhibiting the adenine nucleotide translocase,[42] an effect that reportedly leads to the overproduction of ROS.[43] Detoxification of ROS by a virus-encoded GPX may antagonize apoptosis in infected cells, perhaps facilitating viral replication. Indeed, apoptosis inhibition by overexpression of Bcl-2 or caspase inhibition can enhance HIV-1 replication in culture,[44–47] and it is well possible that the HIV-1 GPX exerts a similar effect, in line with the fact that laboratory

strains of HIV-1 tend to possess an intact GPX gene (like the HIV-1^{LAI} isolated shown in Figure 1).

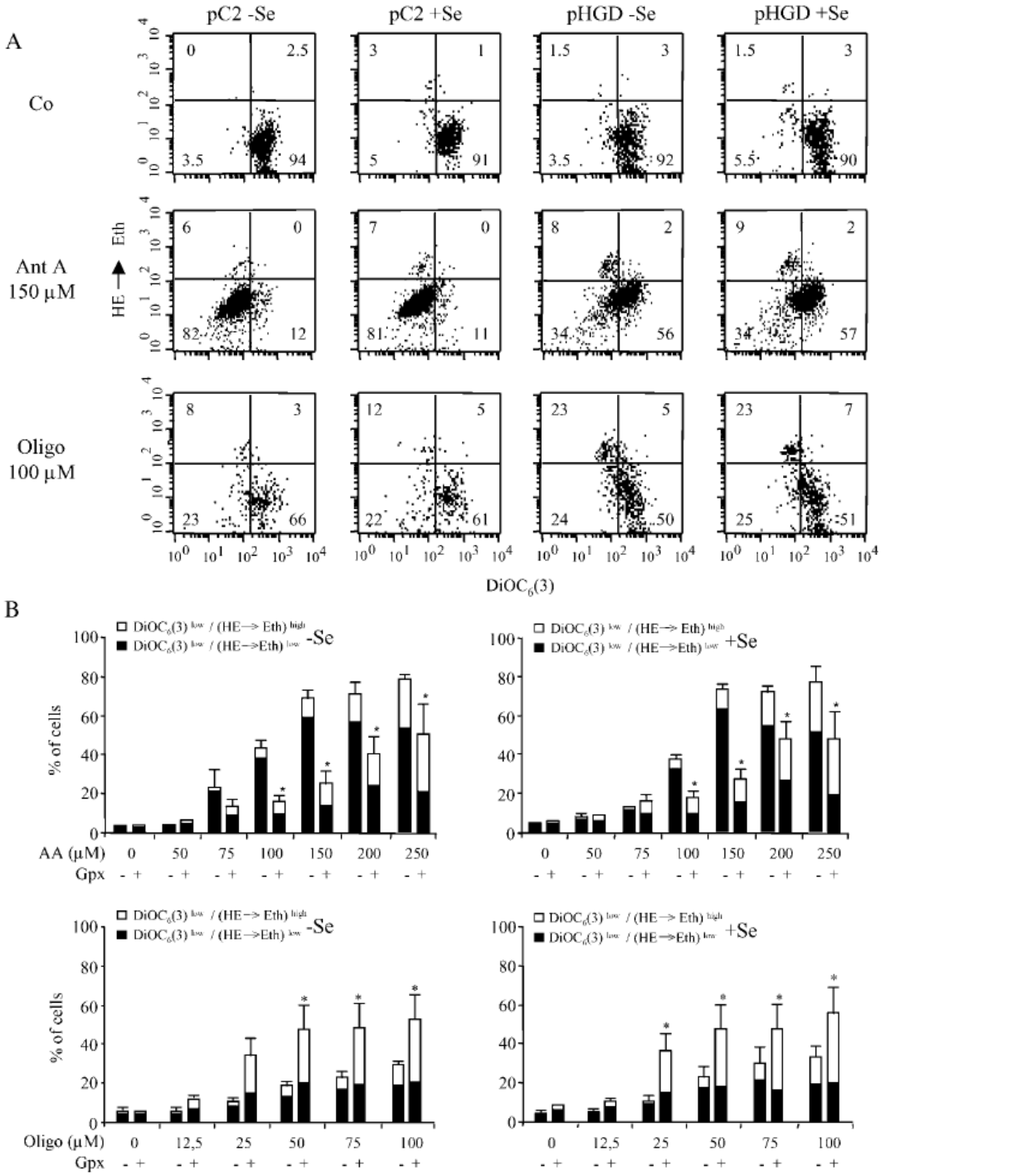

Figure 6. HIV-1 GPX confers resistance against apoptosis induced by mitochondrial ROS. MDCK cells stably transduced with the control vector (pC2) or the HIV-1 GPX construct (pHGD) were cultured in the absence or presence of Se and exposed to the indicated doses of antimycin A or oligomycin for 18 h followed by staining with the $\Delta\Psi_m$-sensitive dye DiOC6(3) and the ROS-sensitive dye HE. Representative FACS diagrams are shown in A, and mean values (X±SEM) from five different experiments are shown in B. Asterisks denote a significant protective effect conferred by HIV-1 GPX.

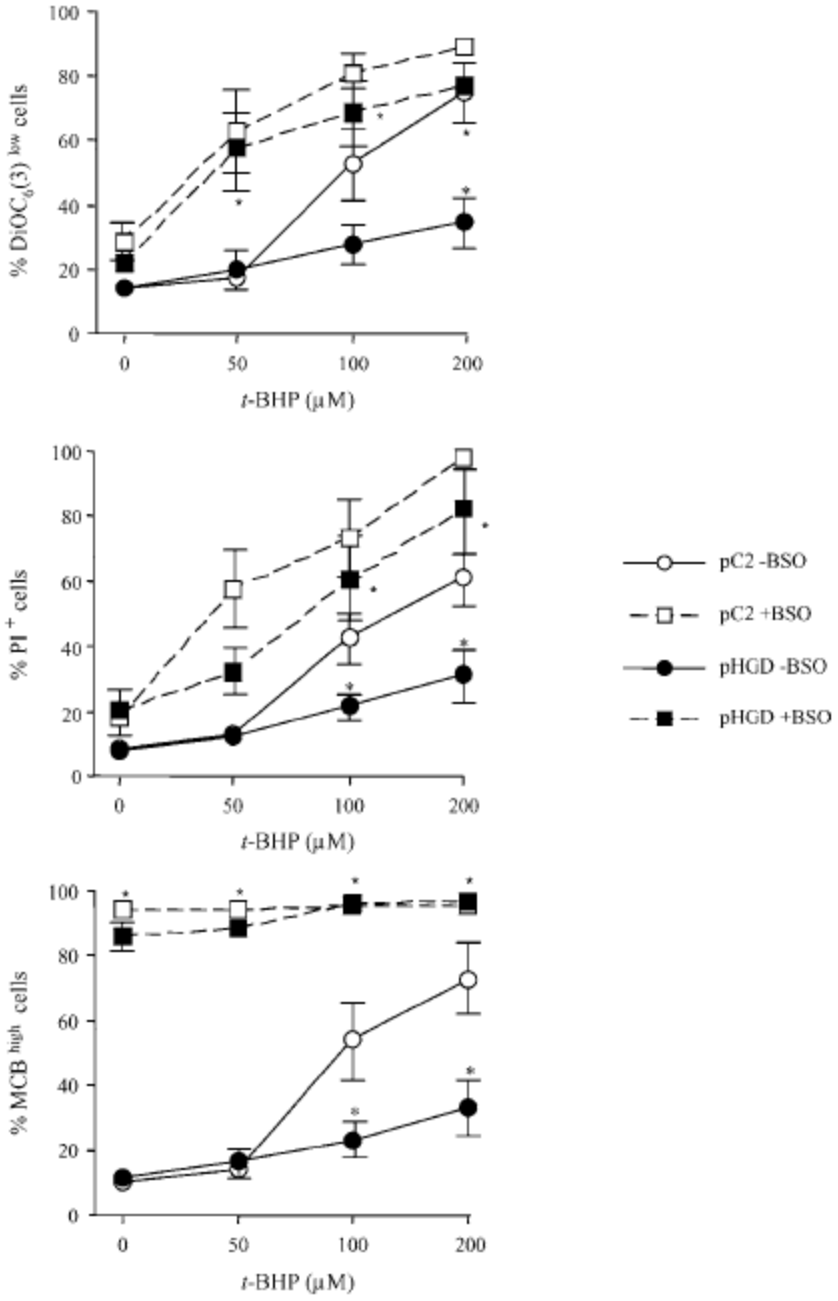

Figure 7. Effect of GSH depletion on cell death induced by *t*-BHP. Control (pC2) or HIV-1 GPX-transfected cells (pHGD) were pretreated with buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, for 24 h and then exposed to the indicated dose of *t*-BHP. After a further incubation period of 18 h, the frequence of cells with a low $\Delta\Psi_m$ (determined by staining with $DiOC_6(3)$) and a permeable plasma membrane (determined by staining with PI) was assessed by cytofluorometry. In addition, the degree of GSH depletion was measured with monochlorobiman (MCB), which emits fluorescence upon formation of GSH adducts. This experiment was repeated three times with comparable results.

It remains on ongoing conundrum which are the optimal electron acceptors used by HIV-1 GPX. In BSO-treated cells depleted from GSH (as determined by MCB staining), HIV-1 GPX continues to be an efficient antiapoptotic factor. This suggests that HIV-1 GPX may transfer electron on other acceptors such as thioredoxin or redox-active disulfides, as this has been shown

for several human GPX isoforms.[48,49] Alternatively, the K_d of HIV-1 GPX for GSH might be that low that trace amounts of GSH would be sufficient to maintain its ROS-detoxifying role. However, mammalian GPX have a K_d for GSH in the range of 10^{-5} to 10^{-4} M,[50] which argues against this latter possibility. However, it appears that the overall GPX fold and the selenocysteine play an essential role in the anti-apoptotic function of GPX since mutations of structural residues (such as P55S), stop mutations within R2 (such as Q52STOP), and removal of the SECIS sequence (essential for the synthesis of the selenocysteine) destroy the anti-apoptotic function of HIV-1GPX(Ref. [32] and data not shown).

That the HIV-1 GPX is essential for viral infection, at some stage, is suggested by the fact that the protein is well conserved among different virus strains, among LTNP. Our data indicate that, among different HIV-1 strains from LTNP, the third nucleotide within each codon of the Env gene (hence the first nucleotide of each triplet coding for HIV-1 GPX) is much more conserved within the region coding for HIV-GPX (with only 1.67 ± 0.15% [X ± SEM] nucleotide exchanges among the sequences shown in Figure 1) than outside of this region (3.43 ± 0.33%, $p < 0.001$, paired Student t test). Given the notorious propensity of HIV-1 to mutate, the only explanation for such a degree of conservation is its vital role for HIV-1 infection, at some stage of the viral life cycle. In contrast, in patients developing AIDS, this difference in conservation disappeared, with 4.33 ± 0.47% exchanges of the third nucleotide in the region of the Env gene corresponding to HIV-GPX versus 4.77 ± 0.43% exchanges outside this region (data for the HIV-1 isolates in Figure 1, excluding the hypermutated isolates AJ302646, AJ0006022 and L20571). It appears paradoxical that HIV-1 isolates from LTNP tend to have a more conserved GPX gene than HIV-1 isolated from individuals developing AIDS. As a possible explanation, loss-of-function of GPX may increase the apoptosis-inducing potential of HIV-1, thereby contributing to terminal lymphodepletion. Thus, as a tantalizing mirror image, the pro-apoptotic function of the Vpr protein would be maintained during the progression to AIDS (yet inactivated in LTNP), while the anti-apoptotic function of GPX would be lost in progressive disease (and conserved in LTNP).

Acknowledgments

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